Reference: *Biol. Bull.*, 138: 26–34. (February, 1970)

# DUAL CONTROL OF THE LEUCOPHORES IN THE PRAWN, *PALAEMONETES VULGARIS*, BY PIGMENT-DISPERSING AND PIGMENT-CONCENTRATING SUBSTANCES [1]

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Examination of the literature dealing with the leucophores of the prawn, *Palaemonetes vulgaris*, reveals that Perkins and Snook (1932) reported that the eyestalks of this organism contain a substance which causes dispersion of its white chromatophoric pigment whereas later Brown (1935) and Hanström (1937) using the same species came to the opposite conclusion, namely that the eyestalk contains a white pigment-concentrating substance. Both Brown and Hanström suggested that dispersion of the white pigment was due to absence of the pigment-concentrating substance. More recently, however, Fingerman and Rao (1969) showed that the eyestalks of *Palaemonetes vulgaris* contain a substance that disperses the white chromatophoric pigment of the fiddler crab, *Uca pugilator*, and another that concentrates this pigment in the crab. But whether these substances would have corresponding actions on the leucophores of *Palaemonetes vulgaris* itself was still a matter of conjecture. Therefore, in view of the results of Fingerman and Rao (1969) and the seemingly conflicting reports of the earlier investigators, it seemed worthwhile to attempt a re-investigation of the endocrine control of the leucophores in this prawn itself.

## Materials and Methods

The specimens of *Palaemonetes vulgaris* were collected in the vicinity of Woods Hole, Massachusetts by members of the Supply Department at the Marine Biological Laboratory. I wish to express my appreciation for their efforts. In the laboratory the prawns were maintained in aquaria equipped with running sea water.

Earlier efforts in this laboratory to study the substances controlling migration of the white pigment in this prawn had not been fruitful because of difficulties in designing a satisfactory assay system. In order to accomplish the aim of this investigation it was imperative to devise an assay system that would provide reproducible data. The Hogben and Slome (1931) system of staging chromatophores was employed for quantifying the responses of the leucophores in the epidermis adhering to the portion of the carapace dorsal to the heart. These were the cells whose responses would be investigated. According to the Hogben and Slome scheme, stage 1 represents maximal concentration of the pigment, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. In his study Brown (1935) had found that the mean stage of the leucophores in eye-

[1] This investigation was supported by Grant GB-7595X from the National Science Foundation.

stalkless prawns was not a uniform value, but instead covered the entire range from 1 to 5. In the search for an adequate assay system to use in the present experiments it was seen that of 165 eyestalkless prawns kept in white containers under an incident illumination of 754 meter-candles for one hour, 22 prawns had a mean leucophore stage of 1, 33 stage 2, 69 stage 3, 27 stage 4, and 14 stage 5, An attempt was made to utilize eyestalkless prawns with their leucophores in stage 3 for the assays because by using such prawns the presence of both pigment-dispersing and -concentrating substances might be detected. Preliminary experiments soon revealed that such an assay system would indeed be suitable. Different groups of eyestalkless prawns selected on the basis of having a mean leucophore stage of 3 responded to the same extract in a very similar manner. Consequently, more detailed experiments were designed and performed with eyestalkless prawns in white containers under an incident illumination of 754 meter-candles and whose leucophores had a mean of stage 3 as the assay animals. A constant intensity of illumination was important. Brown, Sandeen, and Webb (1948) have previously found that the white pigment of *Palaemonetes* shows an increased degree of dispersion with increased illumination.

Extracts to be injected directly into the prawns were prepared in sea water diluted to the osmotic concentration of the prawn's blood (Fingerman and Connell, 1968) which is equivalent to 61.3% of sea water having an original salinity of 35‰. In those experiments where extracts were chromatographed on the gel Sephadex LH-20 prior to assay either 50 eyestalks or 50 supraesophageal ganglia with the circumesophageal connectives attached were first extracted in 0.8 ml ethanol, centrifuged for three minutes at 1815 × *g* and at 24° C, and then the supernate was applied to the top of the column. The column of Sephadex LH-20 was equilibrated with ethanol which was used as the solvent. The size of the column was 1.5 × 28.0 cm and the void volume was 21 ml. Two milliliter fractions were collected. The flow rate was 30 ml per hour. The alcohol in each fraction was allowed to evaporate and the residue was dissolved in 0.2 ml of the diluted sea water. The injected dose was 0.02 ml.

When extracts of fresh tissues were prepared they were assayed on three or five prawns, as will be described below, but in the chromatography experiments each sample was always assayed on three prawns. For the preparation of extracts directly in isosmotic sea water the desired number of organs was triturated in sufficient saline to provide the desired concentration and then centrifuged under the same conditions as were the ethanol extracts.

Responses to the material obtained after chromatography of the several extracts were expressed in units of the Standard Integrated Response (SIR) as defined by Fingerman, Rao, and Bartell (1967). Calculation of the Standard Integrated Response takes into account both the amplitude and duration of the response.

## Experiments and Results

### *Responses to eyestalk extracts*

The object of the first set of experiments was to determine the responses of eyestalkless prawns whose leucophores had a mean Hogben and Slome stage

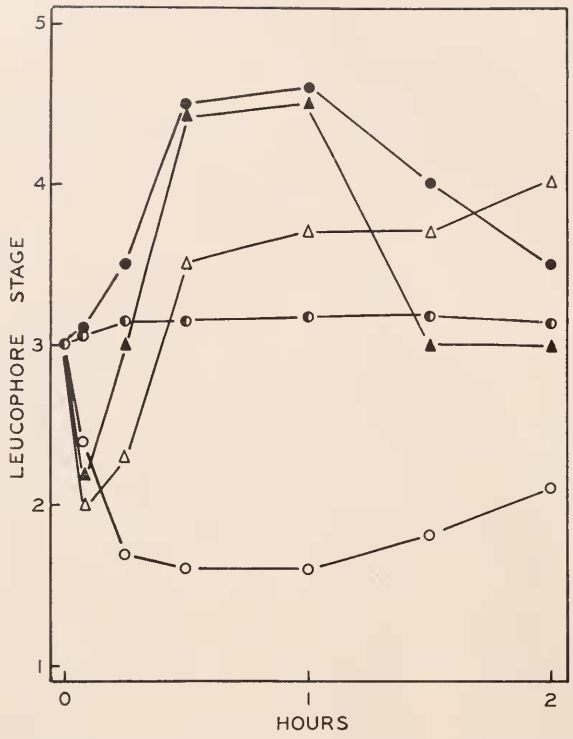


FIGURE 1. Relationships between the mean leucophore stage of eyestalkless prawns injected with extracts prepared from 8 or 16 eyestalks versus time following injection. Half-filled circles, control; other symbols, mean responses of groups of prawns that received an injection of eyestalk extract. A different extract was injected into each group of prawns. Final extract concentrations is: one-half eyestalk equivalent per dose.

of 3 to extracts of eyestalks in an attempt to resolve the apparently incompatible reports of the effect of these extracts. Eyestalks from 4–8 prawns were triturated in sufficient saline to yield an extract containing one-half of an eyestalk equivalent per dose of 0.02 ml. When four such extracts were assayed, each on five eyestalkless prawns, the effects (Fig. 1) ranged from pigment concentration alone to pigment dispersion alone with the intermediate condition of transitory concentration of the white pigment followed by a pigment-dispersing response. The controls received isosmotic sea water alone.

A possible interpretation of the transitory concentration of the white pigment which was followed by dispersion was that because the extracts were prepared from 8–16 eyestalks in sufficient saline to provide a final concentration of one-half eyestalk equivalent per dose, some eyestalks if extracted singly would evoke only a white pigment-dispersing response while others only a white pigment-concentrating response, and that the mixed response was the result of mixing the two types of eyestalks. To determine whether this interpretation might be the correct one 13 extracts were prepared, each from one eyestalk from 13 different prawns. Each eyestalk was triturated in 0.1 ml isosmotic sea water, to provide a final

extract concentration of one-fifth of an eyestalk equivalent per dose. Every one of the 13 extracts was assayed on three eyestalkless prawns. The reason for assaying these extracts on three prawns whereas the previous extracts were assayed on five was that individual eyestalks extracted in only 0.1 ml of fluid did not provide sufficient material for assays on a larger number of prawns because of small losses that occur when extracts are prepared and injected such as, for example, the small volume that always remains in the syringe and needle. The data for three of these 13 extracts are shown in Figure 2. The similarity of the data in Figures 1 and 2 justifies the use of the smaller number of prawns. The results were essentially the same as found with the extracts prepared from more than one eyestalk, some extracts produced dispersion alone, some concentration alone, and some a mixed response. With extracts of single eyestalks the largest responses showing pigment dispersion or concentration alone (Fig. 2) were less than those seen with the extracts prepared from several eyestalks (Fig. 1), reflecting the difference in extract concentration, one-fifth *versus* one-half of an eyestalk equivalent. The results for the entire 13 extracts showed that two produced dispersion alone, four concentration alone, and seven a mixed response. The original hypothesis that the mixed response was due to combining eyestalks that produced either

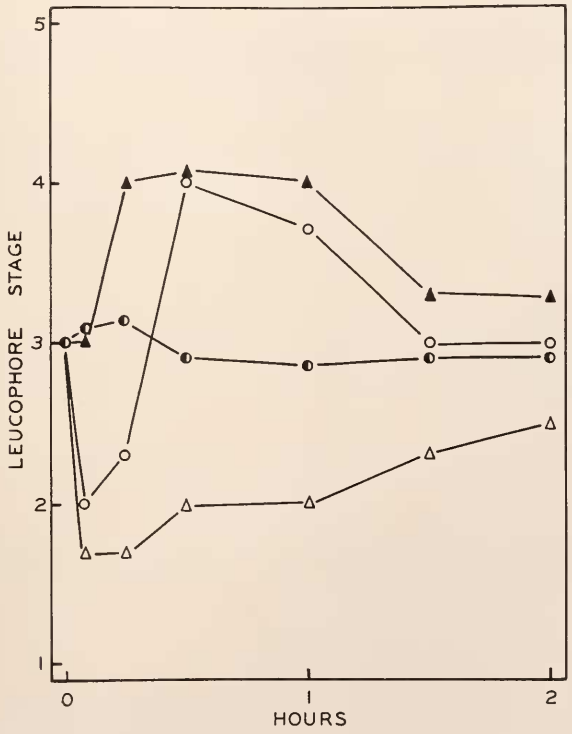


FIGURE 2. Relationships between the mean leucophore stage of eyestalkless prawns injected with extracts prepared from a single eyestalk *versus* time following injection. Symbols same as in Figure 1. Final extract concentration is: one-fifth eyestalk equivalent per dose.

pigment dispersion or concentration alone was not supported by the data with extracts prepared from individual eyestalks.

An experiment utilizing gel filtration on Sephadex LH-20 was then attempted to see if the dispersing and concentrating substances for the white pigment of *Palaemonetes* could be separated from each other by this technique. The averaged results of the experiment, performed three times, are shown in Figure 3. The white pigment-dispersing substance came off the column ahead of the white pigment-concentrating substance, the former peaking in fraction 15 and the latter in fraction 20.

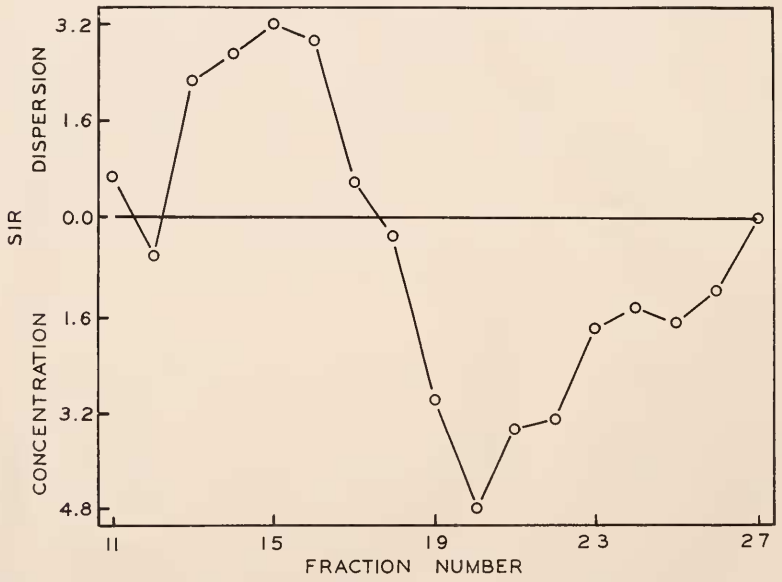


FIGURE 3. The white pigment-dispersing and -concentrating Standard Integrated Responses (SIR) evoked by the fractions obtained by passing the ethanol-soluble material of the eyestalk through the column of Sephadex LH-20.

*Responses to extracts of the supraesophageal ganglia with the circumesophageal connectives attached*

The object of this group of experiments was to compare the responses to extracts of the supraesophageal ganglia with the circumesophageal connectives attached with the results presented above for the eyestalks. The first experiment consisted of a determination of the responses of extracts of freshly dissected supraesophageal ganglia plus the circumesophageal connectives in isosmotic sea water. Extracts were prepared from groups of organ complements in a final concentration of one-half organ equivalent (assayed on five prawns) and from single organ complements having a final concentration of one-fifth of an organ equivalent (assayed on three prawns). The experiment was performed four times, always with the same result. Each extract produced only dispersion of the white pigment. The averaged results of the four experiments are presented in Figure 4. The

effect of the lower final concentration (one-fifth of an equivalent) of material in the extracts from single organ complements is reflected in the lower response to these extracts compared to the response to the extracts (one-half equivalent) prepared from four organ complements.

One possible interpretation of the results in Figure 4 is that the supraesophageal ganglia and circumesophageal connectives contain a white pigment-dispersing substance to the exclusion of white pigment-concentrating material. An alternative explanation is that a white pigment-concentrating substance is present in these organs, but is completely masked by the white pigment-dispersing substance. The technique of gel filtration on Sephadex LH-20 was employed in an effort to dis-

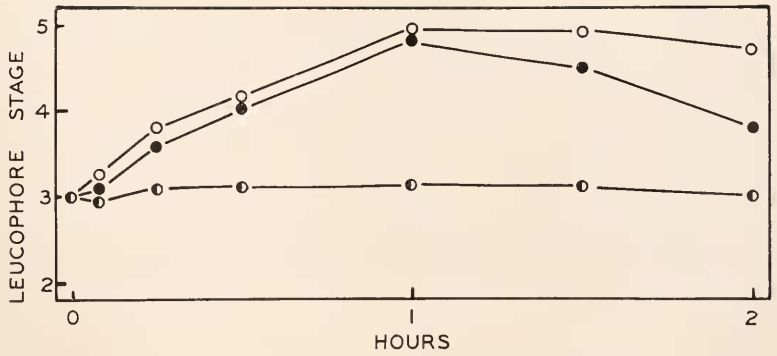


FIGURE 4. Relationships between the mean leucophore stage of eyestalkless prawns injected with extracts of the supraesophageal ganglia with the circumesophageal connectives attached *versus* time following injection. Half-filled circles, control; dots, extracts prepared from single organ complement; circles, extracts prepared from four organ complements. Final extract concentrations are: dots, one-fifth organ equivalent; circles, one-half organ equivalent.

tinguish between these possibilities. A white pigment-concentrating substance was indeed found in addition to the pigment-dispersing one. The experiment was performed three times. The averaged SIR's for the fractions assayed in the three experiments are shown in Figure 5. The peak for the white pigment-concentrating substance in the latter figure occurred in the same fraction as did that in Figure 3 for the eyestalks, fraction 20. The peak of white pigment-dispersing activity occurred in fraction 16 with the supraesophageal ganglia and circumesophageal connectives but in fraction 15 with the eyestalks. However, because the peaks of white pigment-dispersing activity are fairly broad in Figures 3 and 5 there is no significant reason for assuming that the substances are not the same.

## DISCUSSION

The results presented in Figures 1 and 2 showing that some eyestalk extracts produced only dispersion of the white pigment while others produced only concentration provide an explanation for the apparently inconsonant reports of Perkins and Snook (1932) who, as stated above, reported on the one hand that eyestalk extracts caused dispersion of the white pigment of *Palaemonetes* and of Brown

(1935) and Hanström (1937) on the other who reported that these extracts caused concentration. It is conceivable that by chance alone these investigators obtained eyestalks which showed only one effect. The results presented above show clearly that this prawn produces for its own white chromatophoric pigment substances having pigment-dispersing and pigment-concentrating activities. Presumably, all the eyestalks contain both substances but in differing amounts. These substances are certainly antagonistic to each other. The results from the experiment of gel filtration of the supraesophageal ganglia with the circumesophageal connectives attached (Fig. 5) revealed that this antagonism can be strong enough to inhibit completely the response to one of the substances.

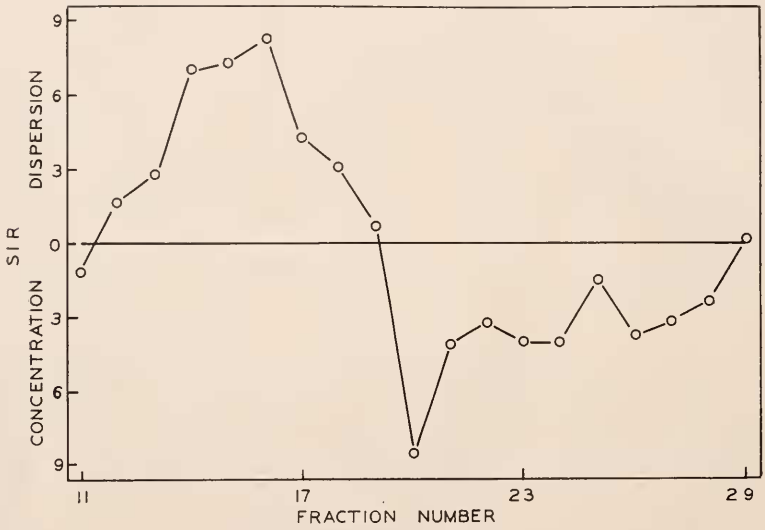


FIGURE 5. The white pigment-dispersing and -concentrating Standard Integrated Responses (SIR) of eyestalkless prawns evoked by the fractions obtained by passing the ethanol-soluble material of the supraesophageal ganglia with the circumesophageal connectives attached through the column of Sephadex LH-20.

Fingerman and Rao (1969), as stated above, found that the eyestalks of this prawn contain substances that disperse and concentrate the white pigment of the fiddler crab, *Uca pugilator*. These investigators chromatographed the ethanol-soluble material from eyestalks of *Palaemonetes* on a column of Sephadex LH-20 having the same dimensions and under the same conditions as employed in the present investigation, and also collected 2 ml fractions as above. The substance that dispersed the white pigment of the fiddler crab peaked in fraction 15 whereas the substance that concentrated the white pigment of this crab peaked in fraction 20, the same two fractions of the eyestalks that had the maximal corresponding effects on the white pigment of *Palaemonetes* (Fig. 3). It would seem most simply then that the corresponding effects produced by the fractionated extracts of eyestalks from *Palaemonetes* in the prawn and crab were due to the same substances. Fingerman and Rao (1969) had, however, found that although dispersion

of the white pigment in the fiddler crab could have been due to the same substance being present in the ethanol-soluble material from the eyestalks of both the crab and the prawn, the substances in the two extracts that concentrated the crab's white pigment were different from each other. The substance in the ethanol-soluble material from the crab's eyestalks that concentrated its white pigment was a larger molecule than the substance from the eyestalk of the prawn that concentrated the white pigment of the fiddler crab, although not as large as the white pigment-dispersing substance.

The conclusion of Brown (1935) and Hanström (1937) that dispersion of the white chromatophoric pigment in *Palaemonetes* was due not to a white pigment-dispersing substance, but merely to the absence of white pigment-concentrating hormone is not supported by the data presented above which revealed that the prawn possesses both substances. From a comparative viewpoint it is interesting that whereas the white pigment-dispersing and -concentrating responses evoked by the fractionated extracts of eyestalks from *Palaemonetes* in *Palaemonetes* (Fig. 3) and *Uca* (Fingerman and Rao, 1969) would appear to have been due to the same substances, Fingerman and Couch (1967) in comparing the responses of the erythrophores of these two crustaceans to aqueous extracts of freshly dissected organs from *Palaemonetes* concluded that the substances that dispersed and concentrated the red pigment in *Palaemonetes* were different from those in *Palaemonetes* that had corresponding effects in *Uca*.

## Summary

1. The prawn, *Palaemonetes vulgaris*, produces substances that cause dispersion and concentration of its white chromatophoric pigment.

2. Injection of extracts prepared from one or more eyestalks triturated directly in isosmotic sea water produced concentration of the white pigment alone, dispersion alone, or concentration followed by dispersion. In contrast, similarly prepared fresh extracts of the supraesophageal ganglia with the circumesophageal connectives attached always produced white pigment dispersion alone.

3. Chromatography of ethanol extracts of eyestalks and as well as supraesophageal ganglia with the circumesophageal connectives attached on the gel Sephadex LH-20 always yielded both the white pigment-dispersing substance and the -concentrating substance. The former preceded the latter off the gel column.

4. The responses obtained with extracts prepared directly in isosmotic sea water were interpreted as having been due to the relative quantities of the white pigment-dispersing and -concentrating substances present in the organs in addition to an antagonism between these chromatophorotropins.

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